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Phage ϕ29 regulatory protein p4 stabilizes the binding of the RNA polymerase to the late promoter in a process involving direct protein–protein contacts

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ABSTRACT **Transcription from the late promoter, P_{A3}, of *Bacillus subtilis* phage ϕ29 is activated by the viral regulatory protein p4. A kinetic analysis of the activation process has revealed that the role of protein p4 is to stabilize the binding of RNA polymerase to the promoter as a closed complex without significantly affecting further steps of the initiation process. Electrophoretic band-shift assays performed with a DNA fragment spanning only the protein p4 binding site showed that RNA polymerase could efficiently retard the complex formed by protein p4 bound to the DNA. Similarly, when a DNA fragment containing only the RNA polymerase-binding region of P_{A3} was used, p4 greatly stimulated the binding of RNA polymerase to the DNA. These results strongly suggest that p4 and RNA polymerase contact each other at the P_{A3} promoter. In the light of current knowledge of the p4 activation mechanism, we propose that direct contacts between the two proteins participate in the activation process.**

The late genes of *Bacillus subtilis* phage ϕ29 are clustered in the central part of its linear genome and are transcribed from a single promoter, named P_{A3}, located in close proximity to the divergent main early promoter, P_{A2b} (1). The late promoter, P_{A3}, is devoid of a −35 consensus sequence for the major *B. subtilis* vegetative σ^A–RNA polymerase and requires the presence of the viral early protein p4 for efficient transcription (2). Protein p4 is a transcriptional regulator that binds to DNA between positions −56 and −102 relative to the P_{A3} transcription start site (2), recognizing an 8-base-pair (bp) inverted repeat that partially overlaps with the −35 region of the early promoter, P_{A2b} (3). This DNA region has an intrinsic curvature that increases considerably when p4 binds to it (refs. 2 and 4; Fig. 1). Since the p4 binding site also contains part of P_{A2b}, activation of P_{A3} is paralleled by repression of P_{A2b} (5). Binding of p4 and RNA polymerase to P_{A3} is strongly cooperative (3), and the activating function requires a precise stereospecific alignment between the two proteins (6), suggesting that direct contacts between p4 and RNA polymerase are probably required for P_{A3} activation.

The initiation of transcription is a complex process involving several steps (7–12). RNA polymerase (R) initially binds to the promoter (P) as a closed binary complex (RP_c). Melting of the DNA strands leads to the formation of an open complex (RP_o) which, in the presence of the four NTPs, proceeds to an initial transcribing complex (ITC; ref. 12) that can be temporarily engaged in abortive transcription before escaping as a productive elongating complex. The transcription initiation process can be limited at different stages. Several transcriptional activators have been shown to act by favoring one or several of these rate-limiting steps (13–19). The molecular mechanisms leading to transcription activation are not fully understood. Direct contacts between the activator and the RNA polymerase have been proposed to participate in the activation process (3, 6, 18, 20–28). At the same time, DNA is thought to play an active role in the initiation mechanism by adopting three-dimensional structures that either directly accelerate one of the steps leading to transcription initiation or facilitate the correct stereospecific alignment of the activator and the RNA polymerase (4, 29–33).

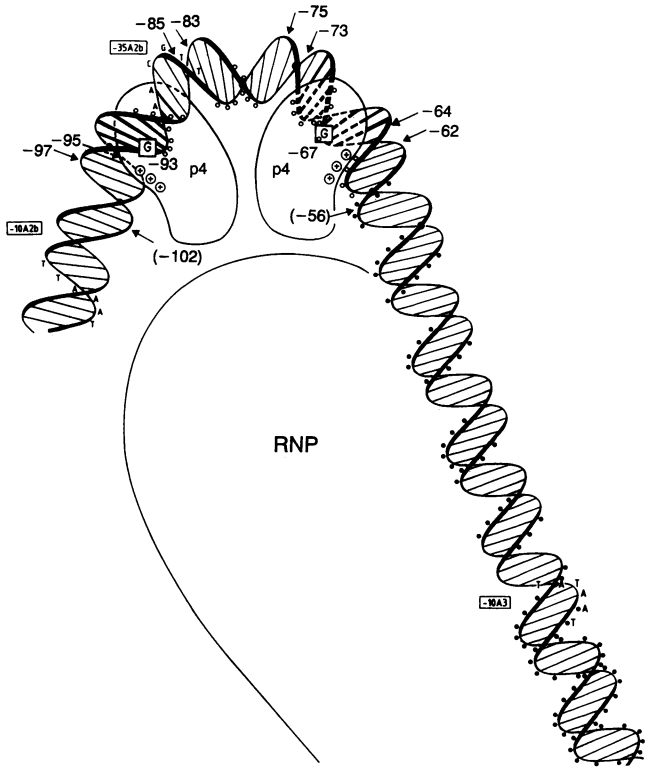

FIG. 1. Spatial representation of the P_{A3} promoter with protein p4 and RNA polymerase (RNP) bound at their respective sites. The inverted repeat recognized by p4 is depicted by thickened base pair lines. The −10 and −35 regions of P_{A2b} and P_{A3}, the positions that become hypersensitive to DNase I cleavage upon binding of protein p4 to DNA (arrows), guanine residues whose methylation interferes with protein p4 binding (enclosed in boxes) and positions that become protected from hydroxyl-radical cleavage by protein p4 (open circles) or by the RNA polymerase in the presence of protein p4 (filled circles) are indicated (2, 4, 5). The DNase I-hypersensitive sites shown in parentheses disappear upon binding of RNA polymerase (unpublished data). Plus signs within protein p4 illustrate the carboxyl end of the protein, which is proposed to be involved in maintaining part of the protein p4-induced DNA bending (4).

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Abbreviations: ITC, initial transcribing complex; RP_o and RP_c, open and closed RNA polymerase–promoter complexes.

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Here we show that the protein p4 transcriptional activator increases RP_c formation at the phage ϕ29 late P_{A3} promoter and we present evidence for direct contacts between p4 and the *B. subtilis* vegetative RNA polymerase.

METHODS

Transcription Assays and Kinetic Analysis. The kinetic parameters of P_{A3} promoter were obtained from τ plots as described (34, 35), except that the reaction was followed by run-off transcription instead of abortive initiation. The 237-bp-long restriction fragment used as template was obtained from pFRC54 (4) with *Pst* I and *Eco*RI, giving rise to a 78-base transcript from P_{A3}. The short length of the run-off transcript analyzed allows us to assume that the lag times observed represent the time required for the RNA polymerase to bind to the promoter and start transcription (36). Reaction conditions were 25 mM Tris·HCl (pH 7.5); 10 mM $MgCl_2$; 92 mM ammonium sulfate; 20 mM NaCl; 200 μM ATP, CTP, and GTP; 80 μM [α-^{32}P]UTP [2 μCi (74 kBq)], and 4% (vol/vol) glycerol. DNA concentration was 0.5 nM and that of protein p4 was 2.6 μM. Incubation was for 10 min at 37°C, and transcription was started by the addition of prewarmed RNA polymerase (8.8 nM–176 nM). After 1, 2, 3, 5, 7, and 9 min, 20-μl aliquots were taken and immediately mixed with 50 μl of a stop solution containing 70 mM EDTA, 0.2% SDS, and 0.2 mg of tRNA per ml. Samples were filtered through 1-ml Sephadex G-50 "spun" columns, precipitated, and electrophoresed in denaturing 6% polyacrylamide gels. The run-off transcript was detected by autoradiography and quantified by laser scanning densitometry. Values were plotted as a time function for each RNA polymerase concentration, showing a linear increase in the amount of product after a lag time. Lag times were calculated as the intersection point with the time axis obtained by extrapolating the linear part of each graph (35). The kinetic constants of each promoter were calculated from the rate equation obtained after a least-squares linear regression analysis of the lag times, plotted vs. the reciprocal of the RNA polymerase concentration.

Run-off assays with the PS restriction fragment (*Taq* I–*Eco*RI, see below) were performed as above, except that template DNA was 4 nM and reaction time was 10 min.

Gel Retardation. The binding sites for protein p4 and RNA polymerase were separated by digesting appropriate restriction fragments with *Taq* I, which cuts at position −56. The restriction fragment containing the *a*ctivator binding *s*ite was named AS, that containing the RNA *p*olymerase binding *s*ite was named PS, and the fragment containing both binding sites was named ASPS. The AS restriction fragment (106 bp, *Hin*dIII–*Taq* I) and the PS fragment (146 bp, *Taq* I–*Eco*RI) were purified from pFRC54 (4). Binding reaction mixtures (20 μl) contained 25 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 2 μg of bovine serum albumin, 1 μg of poly(dI-dC) as competitor DNA, about 1000 cpm of 3′-end-labeled DNA fragment, and 1 μg of protein p4. Incubation was for 10 min at 4°C and for 10 min at room temperature (22°C) before the addition of 0.5 μg of RNA polymerase. Incubation with both proteins proceeded for 10 min more at 4°C or 25°C (as indicated), and the complexes formed were resolved in a 4% polyacrylamide gel (80:1 acrylamide/*N*,*N*′-methylenebisacrylamide weight ratio) at 4°C or at room temperature, respectively. Protein–DNA complexes were visualized by autoradiography.

DNase I Footprinting in the Gel Slice. Binding reactions and polyacrylamide gel electrophoresis to resolve protein–DNA complexes were as indicated above, with 50,000 cpm of labeled DNA per sample. The 71-bp-long DNA fragment used was isolated from the ϕ29 *Hin*dIII-H restriction fragment (759 bp) by treatment with *Acc* I and *Taq* I. The gel was autoradiographed at 4°C and gel slices containing the protein–DNA complexes were cut from the gel and treated with DNase I (18).

Immunological Techniques. The binding reaction mixtures were incubated as above but with 20 ng of unlabeled DNA fragment. The complexes were resolved as before and then transferred onto nitrocellulose membranes overnight at 4°C. Membranes were then saturated with blotting buffer (150 mM NaCl/5 mM EDTA/50 mM Tris·HCl, pH 7.5/0.05% Nonidet P-40/0.25% gelatin/0.01% sodium azide), supplemented with 1% lyophilized skimmed milk. RNA polymerase was detected with polyclonal anti-σ^A antibodies (2) and ^{125}I-labeled protein A.

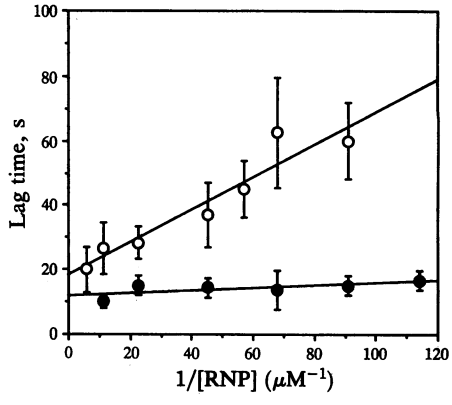
RESULTS AND DISCUSSION

Kinetic Analysis of Phage ϕ29 Late P_{A3} Promoter. The τ plot analysis (34, 35) has been extensively used to determine the kinetic parameters of promoters. This method is based on the observation that, under pseudo-first-order conditions (i.e., a molar excess of RNA polymerase), a transcription reaction that is initiated by addition of the RNA polymerase shows a lag time before the reaction reaches its steady state. This lag time is related to the time required for free enzyme (R) and free promoter (P) to combine and isomerize to start transcription. The process is assumed to be as follows:

$$R + P \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} RP_c \xrightarrow{k_2} RP_o \xrightarrow{k_3} ITC \xrightarrow{k_4} \text{elongating complex.}$$

Plotting the observed lag times vs. the inverse of the enzyme concentration leads to a rate equation in which the slope represents the inverse of the promoter strength ($K_B k_f$; $K_B = k_1/k_{-1}$, reflecting the affinity of the RNA polymerase for the promoter), and the time axis intercept is the inverse of the forward rate constant ($k_f = k_2 k_3 k_4$ when the reaction product analyzed is a run-off transcript). Therefore, under our conditions k_f not only represents the step of RP_o formation but also the rest of the steps leading to the formation of an elongation complex.

We have used this approach to study the behavior of the P_{A3} promoter in the absence and in the presence of the activator p4. Fig. 2 summarizes the kinetic values obtained.



	K_B	k_f	$K_B k_f$	p4 stimulation factor		
				K_B	k_f	$K_B k_f$
-p4	3.6×10^7	5.5×10^{-2}	2.0×10^6	8	1.5	12.5
+p4	2.9×10^8	8.5×10^{-2}	2.5×10^7			

FIG. 2. Kinetic analysis of P_{A3} activation by protein p4. τ plots and kinetic parameters of the P_{A3} promoter in the absence (○) or presence (●) of p4 are shown. K_B (M^{-1}) is the pseudo-first-order association constant reflecting the affinity of RNA polymerase (RNP) for the promoter. The velocity constant k_f (s^{-1}) describes all steps from RP_o formation to promoter clearance. The product $K_B k_f$ ($M^{-1}s^{-1}$) indicates the promoter strength.

The affinity of the RNA polymerase for P_{A3} was rather low ($3.6 \times 10^7\ M^{-1}$), as expected for a promoter devoid of the −35 consensus region, where binding of the RNA polymerase is thought to initiate (37). The presence of p4 increased this affinity 8-fold, while the rest of the steps leading to a stable elongating complex were accelerated as a whole 1.5-fold. Therefore, p4 increased the overall promoter strength ($K_B k_f$) about 12-fold, affecting mainly RP_c formation. This result suggests that p4 might provide alternative signals that enable the recognition and/or stabilization of the RNA polymerase at the promoter in the absence of a −35 consensus sequence.

Direct Contacts Between Protein p4 and RNA Polymerase. To analyze whether protein p4 and RNA polymerase contact each other when bound at the P_{A3} promoter, we took advantage of the *Taq* I restriction site at P_{A3} position −56, between the binding sites for the two proteins (Figs. 1 and 3). This allowed us to separate both binding sites and to search for p4–RNA polymerase complexes when only the p4 or RNA polymerase binding site was present. DNA fragments including only the activator binding site (AS), only the RNA polymerase binding site (PS), or both binding sites (ASPS) were radiolabeled and then incubated with protein p4, RNA polymerase, or both, and the protein–DNA complexes formed were analyzed by gel retardation. When the incubation was carried out at 4°C (Fig. 4*A*) p4 bound only to the fragments (AS and ASPS) containing its binding site. RNA polymerase bound less efficiently to the PS fragment than to the AS and ASPS fragments, probably because the AS and ASPS fragments include the early promoter, P_{A2b}. In fact, binding of the RNA polymerase to an ASPS derivative containing the late P_{A3} promoter and a mutated P_{A2b} promoter produced a single retarded complex less efficiently than when the wild-type ASPS fragment was used (data not shown). Binding of the RNA polymerase to the ASPS fragment produced two different complexes that seemed to reflect its binding to either the P_{A2b} or the P_{A3} promoters, as p4 enhanced the formation of one of them but repressed the formation of the other (Fig. 4*A* and data not shown). When the binding of p4 to the AS fragment was analyzed in the presence of *B. subtilis* RNA polymerase, almost all the DNA appeared as a slowly migrating complex that moved similarly to the complex formed by the RNA polymerase alone. Therefore, this slowly migrating complex seemed to be composed of protein p4 bound at its binding site and complexed somehow with the RNA polymerase. When the assay was performed with the PS fragment, the result obtained was similar: binding of *B. subtilis* RNA polymerase to DNA was stimulated in the presence of p4, despite the fact that there was no p4 binding site. It therefore seemed that both proteins could form complexes at 4°C that were stable enough to be detected by gel retardation. It is interesting that the stabilizing effect of p4 on the RNA polymerase–DNA complex was obtained in a situation where no DNA upstream from the RNA polymerase binding site was provided. Therefore, any stabilizing effect transmitted through DNA can be excluded and the stabilization observed can be attributed to direct protein–protein interactions between p4 and RNA polymerase.

Several control experiments were undertaken to investigate the nature of the above complexes. First, protein p4 was replaced in the assays by a deletion mutant, p4(CΔ14+2), which lacks the last 12 amino acids of its carboxyl end. This mutant protein is unable to activate transcription, although it binds to the p4 recognition sequences (4). This mutant could bind normally to the AS fragment but prevented the formation of slowly migrating complexes (Fig. 4*A*), suggesting that it was even displacing the RNA polymerase bound to P_{A2b}. In fact, this mutant protein is able to repress P_{A2b} upon binding to DNA (F.R. and M.S., unpublished). Mutant protein p4 was also unable to enhance the binding of the RNA

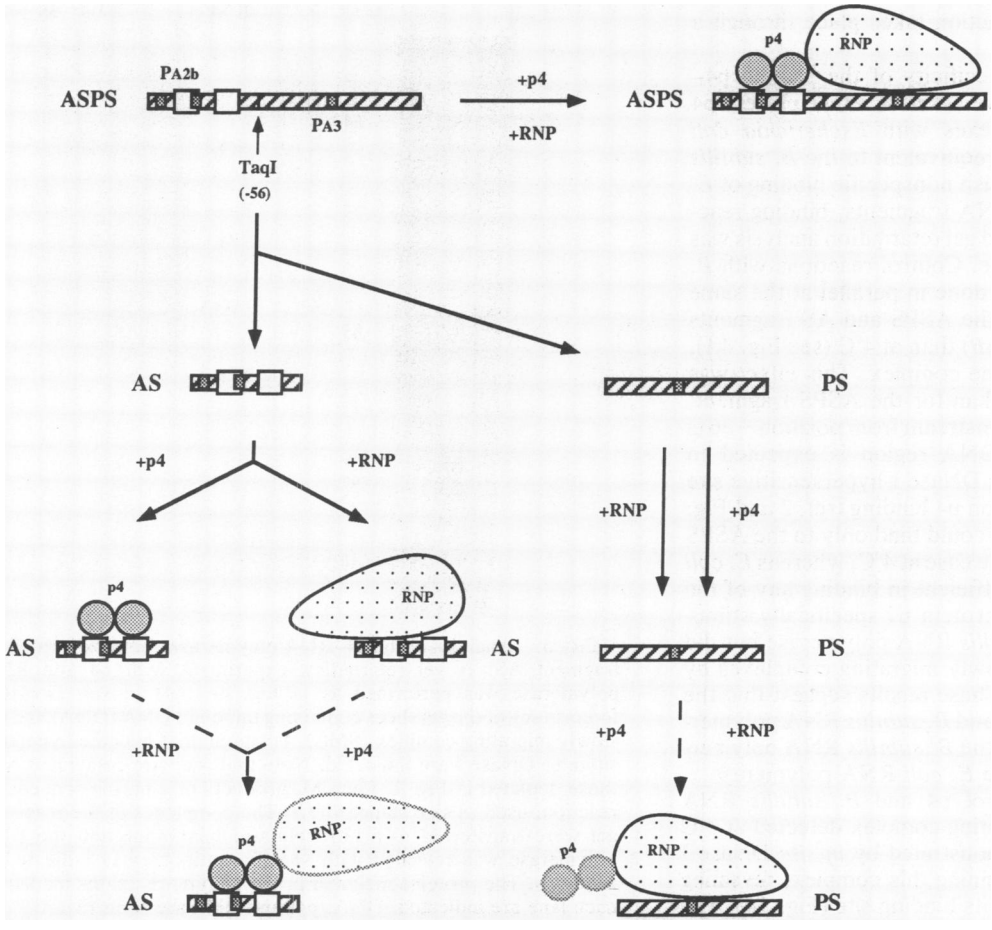


FIG. 3. Strategy used for the analysis of protein p4–RNA polymerase complexes. The *Taq* I restriction site was used to separate p4 and RNA polymerase (RNP) binding sites at the P_{A3} promoter. The activator binding site is indicated as two white boxes. Stippled rectangles show the −10 and −35 boxes for RNA polymerase. Dashed pathways indicate the formation of the putative complexes described in Fig. 4. Proteins held in the complexes by direct protein–protein interactions are shown with dotted outlines.

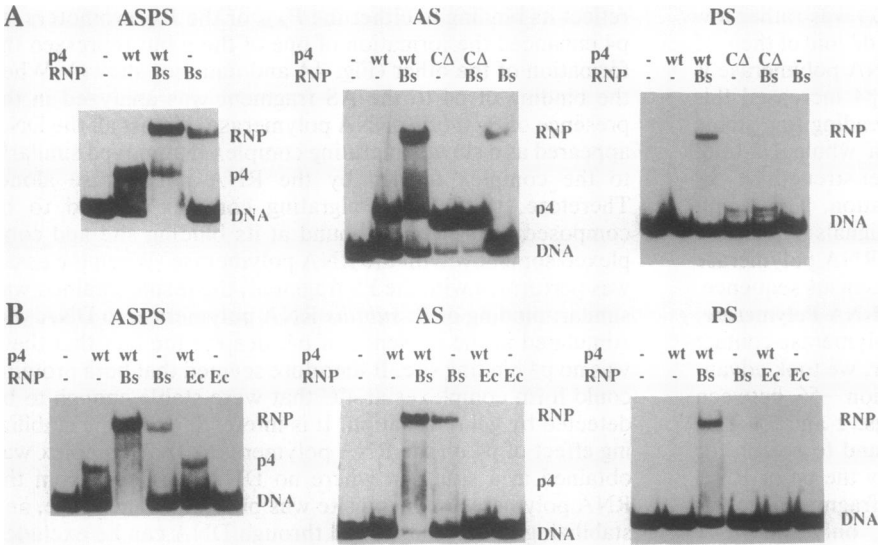


FIG. 4. Complexes formed by protein p4 and RNA polymerase with various DNA fragments. The binding of wild-type protein p4 (wt), its deletion derivative (CΔ), and the RNA polymerase (RNP) of *B. subtilis* (Bs) or of *E. coli* (Ec) to different templates (AS, PS, and ASPS fragments) was analyzed by gel retardation assays. DNA stands for unbound template, and p4 for the p4–DNA complex; both the RNA polymerase–DNA complexes and the slowly migrating complexes in which p4 is also involved are indicated as RNP. AS is the *Hin*dIII–*Taq* I restriction fragment and PS the *Taq* I–*Eco*RI fragment, except in *B*, in which it is *Taq* I–*Bam*HI (see *Methods*). (*A*) Binding of RNA polymerase in the absence or presence of the wild-type protein p4 or its deletion derivative; incubations and gel electrophoresis were performed at 4°C. (*B*) Binding of *B. subtilis* or *E. coli* RNA polymerase in the absence or presence of p4; incubations were at 25°C, and electrophoresis was at room temperature.

polymerase to the PS fragment. The doublet of faint retarded bands that appear with the PS fragment are probably due to minor contaminants of p4(CΔ14+2) (ref. 4 and unpublished observations). The positively charged carboxyl end, which is missing in this mutant, has been shown to be involved in maintaining part of the curvature generated by protein p4 in its binding site and is therefore considered to interact with the DNA (4). The results presented above suggest that the carboxyl end of p4 could also contain residues to interact with the RNA polymerase, which are missing in the deletion mutant. Alternatively, the deletion could alter the folding of this region of the protein in such a way that the complexes can no longer be formed. In either case, this result suggests that the slowly migrating complex observed with the wild-type p4 arises from an interaction between the activator and the RNA polymerase, and that the interaction takes place through a defined region of the protein.

To further investigate the specificity of the protein p4–RNA polymerase interaction, we asked whether protein p4 could also form stable complexes with *Escherichia coli* σ^{70}–RNA polymerase, which is equivalent to the *B. subtilis* σ^{A}–RNA polymerase. To diminish nonspecific binding of *E. coli* RNA polymerase to the DNA fragments, binding reactions were performed at 25°C and gel retardation analysis was carried out at room temperature. Control reactions with *B. subtilis* RNA polymerase were done in parallel at the same temperature. Binding of p4 to the ASPS and AS fragments was less efficient at 25°C (Fig. 4*B*) than at 4°C (see Fig. 4*A*), reflecting a destabilization of the complex. This effect was stronger for the AS fragment than for the ASPS fragment, probably because the DNA downstream from position −56 is missing in the former. This DNA region is expected to interact with protein p4, since a DNase I-hypersensitive site at position −56 is generated upon p4 binding (ref. 2 and Fig. 1). *B. subtilis* RNA polymerase could bind only to the ASPS and the AS fragments, as was the case at 4°C, whereas *E. coli* RNA polymerase was very inefficient in binding any of the tested fragments. Addition of protein p4 specifically stimulated the binding of the *B. subtilis* RNA polymerase but did not induce the formation of slowly migrating complexes by the *E. coli* RNA polymerase. These results suggest that the interaction between protein p4 and *B. subtilis* RNA polymerase occurs through a region of the *B. subtilis* RNA polymerase that is not conserved in the *E. coli* RNA polymerase.

The simultaneous presence of p4 and *B. subtilis* RNA polymerase in the slowly migrating complex detected at 4°C with the AS fragment was demonstrated by *in situ* DNase I footprinting in a gel slice containing this complex, revealing that p4 was correctly bound to its binding site (Fig. 5*A*). The pattern of protected and hypersensitive bands obtained was the well-characterized one generated by p4 in the presence of RNA polymerase (Fig. 1), suggesting that the interactions between p4 and RNA polymerase were being properly held. The presence of RNA polymerase in the complexes formed, both in the absence and in the presence of p4, was revealed by immunological analysis using antibodies against the σ^{A} subunit (Fig. 5*B*). The slight difference in the mobility of the complexes obtained probably indicates that the RNA polymerase had been displaced from the early P_{A2b} promoter and suggests that it was binding the AS fragment through protein p4.

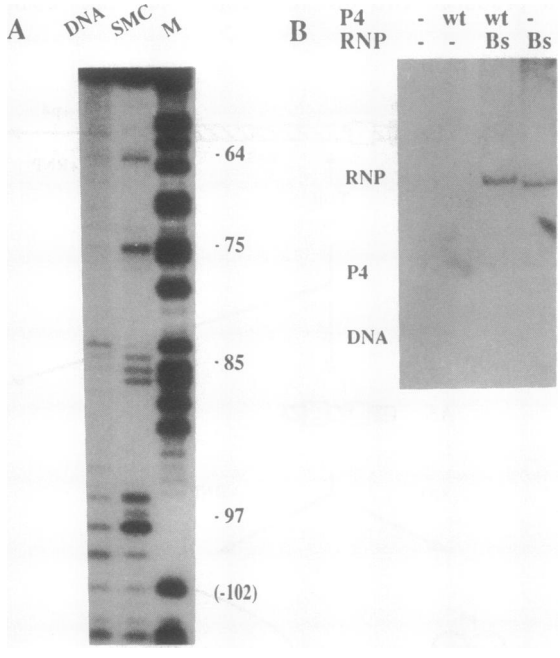


FIG. 5. Analysis of the p4–RNA polymerase complex formed on fragment AS. A gel retardation experiment with p4 and RNA polymerase was performed as in Fig. 4*A*. (*A*) *In situ* DNase I footprinting of the gel slices containing unbound DNA (DNA) and the slowly migrating complex (SMC). Positions that become hypersensitive to DNase I are indicated. Note that they are coincident with those depicted in Fig. 1. Lane M, products of a purine sequencing reaction, used as size standard. (*B*) The complexes of the resolving gel were transferred to a nitrocellulose membrane and the RNA polymerase was revealed with specific antibodies as indicated in *Methods*. The proteins included in the binding reactions loaded on each lane are indicated. DNA, p4, and RNP are as in Fig. 4.

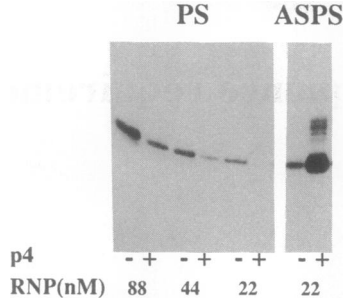


FIG. 6. Effect of protein p4 on P_{A3} transcription in the absence of its binding site. Run-off assays were performed at 22, 44, and 88 nM RNA polymerase (RNP) with the PS (*Taq* I–*Eco*RI) or ASPS (*Pst* I–*Eco*RI) fragment as template.

The effect of protein p4 on the activity of the P_{A3} promoter in the PS fragment was analyzed by run-off experiments using various concentrations of template, RNA polymerase, and activator. Protein p4 moderately repressed transcription from P_{A3} in this template (about 2-fold; Fig. 6) suggesting that although p4 was able to help the binding of the RNA polymerase to the PS fragment (Fig. 4), it hindered the transition from closed to elongation complexes at P_{A3}. This inhibitory effect can be explained by taking into account that, in the PS fragment, p4 is not strongly bound in cis to its recognition site, as in the natural promoter. The putative protein p4–RNA polymerase interactions leading to transcription activation are expected to be easily disrupted when the RNA polymerase leaves the promoter. If p4 is not bound to the DNA, disruption of such interactions might be disfavored, and hence transcription might be inhibited. An observation consistent with our proposal was obtained with the OmpR transcriptional activator, which also stabilizes the binding of the RNA polymerase to the promoter as a closed complex. When the naturally activated weak promoter was artificially replaced by a strong promoter, the positive effect of OmpR turned to inhibitory (13), a result that was interpreted as a strong evidence for the existence of direct contacts between the RNA polymerase and the activator.

Our results show that regulatory protein p4 activates transcription at the ϕ29 late P_{A3} promoter by increasing the affinity of RNA polymerase for the promoter. We have also presented evidence in support of direct protein–protein contacts between the activator and the polymerase. These results, together with the reported cooperativity in the binding of protein p4 and RNA polymerase to P_{A3} (3) and the need for a correct stereospecific alignment of both proteins for transcription activation (6), strongly suggest that direct contacts between p4 and RNA polymerase participate in the activation process. Evidence for the existence of contacts between transcriptional activators and RNA polymerase has been also reported in other systems, based in at least five different observations: (*i*) the availability of point mutations in the regulatory protein that do not affect promoter recognition but abolish the activating function [λ cI, ref. 20; cAMP receptor protein (CRP), refs. 22 and 25]; (*ii*) insertions and deletions in the promoters, indicating that a correct stereospecific alignment of the activator and the RNA polymerase is required (OmpR, ref. 26; CRP, refs. 18, 23, and 24); (*iii*) the strong cooperativity observed in the binding of some activators and the RNA polymerase to the promoter (CRP, refs. 18 and 21); (*iv*) the isolation of mutations in the RNA polymerase which do not respond to transcription activation by certain activators but transcribe normally from many other promoters (27); and (*v*) direct visualization under the electron microscope of DNA loops mediated by NtrC and the minor σ^{54}–RNA polymerase (28). The increase in DNA curvature produced by protein p4 bound in cis at its binding site is also likely to participate in the activation process, not only creating a structure that might stabilize the RNA polymerase on the promoter but also ensuring the correct stereospecific alignment between the activator and the RNA polymerase to allow productive contacts between them.

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1. Mellado, R. P., Barthelemy, I. & Salas, M. (1986) *J. Mol. Biol.* **191**, 191–197.
2. Barthelemy, I. & Salas, M. (1989) *J. Mol. Biol.* **208**, 225–232.
3. Nuez, B., Rojo, F., Barthelemy, I. & Salas, M. (1991) *Nucleic Acids Res.* **19**, 2337–2342.
4. Rojo, F., Zaballos, A. & Salas, M. (1990) *J. Mol. Biol.* **211**, 713–725.
5. Rojo, F. & Salas, M. (1991) *EMBO J.* **10**, 3429–3438.
6. Serrano, M., Barthelemy, I. & Salas, M. (1989) *J. Mol. Biol.* **219**, 403–414.
7. Buc, H. & McClure, W. R. (1985) *Biochemistry* **24**, 2712–2723.
8. Straney, D. C. & Crothers, D. C. (1987) *J. Mol. Biol.* **193**, 267–278.
9. Straney, D. C. & Crothers, D. C. (1987) *J. Mol. Biol.* **193**, 279–292.
10. Schickor, P., Metzger, W., Werel, W., Lederer, H. & Heumann, H. (1990) *EMBO J.* **9**, 2215–2220.
11. Chan, B., Minchin, S. & Busby, S. (1990) *FEBS Lett.* **267**, 46–50.
12. Krummel, B. & Chamberlin, M. J. (1989) *Biochemistry* **28**, 7829–7842.
13. Tsung, K., Brissette, R. E. & Inouye, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5940–5944.
14. Popham, D. L., Szeto, D., Keener, J. & Kustu, S. (1989) *Science* **24**, 629–635.
15. Morett, E. & Buck, M. (1989) *J. Mol. Biol.* **210**, 65–77.
16. Hawley, D. K. & McClure, W. R. (1982) *J. Mol. Biol.* **157**, 493–525.
17. Herbert, M., Kolb, A. & Buc, H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2807–2811.
18. Straney, D. C., Straney, S. B. & Crothers, D. M. (1989) *J. Mol. Biol.* **206**, 41–57.
19. Menendez, M., Kolb, A. & Buc, H. (1987) *EMBO J.* **6**, 4227–4234.
20. Bushman, F. D., Shang, C. & Ptashne, M. (1989) *Cell* **58**, 1163–1171.
21. Ren, Y. L., Garges, S., Adhya, S. & Krakow, J. S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4138–4142.
22. Bell, A., Gaston, K., Williams, R., Chapman, K., Kolb, A., Buc, H., Minchin, S., Williams, J. & Busby, S. (1990) *Nucleic Acids Res.* **18**, 7243–7250.
23. Gaston, K., Bell, A., Kolb, A., Buc, H. & Busby, S. (1990) *Cell* **62**, 733–743.
24. Ushida, C. & Aiba, H. (1990) *Nucleic Acids Res.* **18**, 6325–6330.
25. Eschenlauer, A. C. & Reznikoff, W. (1991) *J. Bacteriol.* **173**, 5024–5029.
26. Maeda, S., Ozawa, Y., Mizuno, T. & Mizushima, S. (1988) *J. Mol. Biol.* **202**, 433–441.
27. Igarashi, K., Hanamura, A., Makino, K., Aiba, H., Aiba, H., Mizuno, T., Nakata, A. & Ishihama, A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8958–8962.
28. Su, W., Porter, S., Kustu, S. & Echols, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5504–5508.
29. Bracco, L., Kotlarz, D., Kolb, A., Diekmann, S. & Buc, H. (1989) *EMBO J.* **8**, 4287–4296.
30. McAllister, C. F. & Achberger, E. C. (1989) *J. Biol. Chem.* **264**, 10451–10456.
31. Gartenberg, M. R. & Crothers, D. M. (1991) *J. Mol. Biol.* **219**, 217–230.
32. Leirmo, S. & Gourse, R. L. (1991) *J. Mol. Biol.* **220**, 555–568.
33. Lavigne, M., Herbert, M., Kolb, A. & Buc, H. (1992) *J. Mol. Biol.* **224**, 293–306.
34. McClure, W. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5634–5638.
35. Hawley, D. K. & McClure, W. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6381–6385.
36. McClure, W. R. (1985) *Annu. Rev. Biochem.* **54**, 171–204.
37. Mecsas, J., Cowing, D. W. & Gross, C. A. (1991) *J. Mol. Biol.* **220**, 585–597.